bio-protocol Preprint

Not Peer Reviewed
Ask a question online

AAV vector production

Sean K Wang Constance L. Cepko

Updated date: Jun 25, 2021

An abbreviated version of this protocol was published in The Journal of Clinical Investigation in Jul 2020

Microglia modulation by TGF-β1 protects cones in mouse models of retinal degeneration

DOI: 10.1172/JCI136160

Detailed protocol

The following protocol has been optimized for AAV8 vectors. Minor modifications may be needed for other serotypes.
For each AAV vector:

1. Grow five 15-cm plates of 293T cells. Await 80-90% confluency.
2. Mix Helper (100ug), Rep2/Cap8 (35ug), and AAV vector (35ug) plasmids in a 50mL tube.
3. Add 25mL of plain DMEM to the 50mL tube.
4. Add 680ul polyethyleneimine (PEI) transfection reagent to the 50mL tube. Mix by inversion and incubate for 15 min at room temperature.
5. During incubation, replace the media of each plate with 20mL of DMEM + 10% NuSerum + 1% Penicillin-Streptomycin.
6. Add 5mL of transfection mixture to each plate. Rock plates to mix and return to incubator.

24 hours post-transfection:

1. Replace media of each plate with 25mL of DMEM + 1% Penicillin-Streptomycin (no serum).

72 hours post-transfection:

1. Collect media from each plate in 50mL tubes (total volume ~125mL) while minimizing dislodging of cells. Centrifuge media at 600 x g for 10 min to pellet cells.
2. Filter supernatant into a vacuum container with 0.45um pore size.
3. Move collected supernatant to 4 deg and place on a magnetic stir plate.
4. Slowly add 2.92g of NaCl to mixture while stirring for a final concentration of 0.4 M.
5. Slowly add 10.63g of PEG-8000 to mixture for a for a final concentration of 8.5%. Stir overnight.
6. Centrifuge mixture at 7000 x g for 10 min to precipitate proteins including AAV capsids. A pellet may or may not be visible.
7. Resuspend pellet or expected location of pellet in 13.5mL of lysis buffer (see buffers below).
8. Add 13.5uL of benzonase to lysis mixture and incubate at 37 deg for 30 min.
9. During incubation, prepare the iodixanol gradient. In a 36.2mL OptiSeal tube, add in order: 6mL of iodixanol solution A, 6mL of iodixanol solution B, 5mL of iodixanol solution C, and 6mL of iodixanol solution D (see buffers below). Slowly add each to the bottom of the tube while minimizing disruption of layers. We recommend using a 10mL Luer-lock syringe and a blunt-ended Hamilton needle for this.
10. Following benzonase treatment, filter the lysis mixture through a 0.22um membrane.
11. Gently add lysis mixture on top of the iodixanol gradient.
12. Ultracentrifuge the iodixanol gradient at 200,000 x g for 90 min at 16 deg.
13. Using an 18-gauge needle and 5mL Luer-lock syringe, collect 3-4mL of the clear 40% layer (iodixanol solution C) through the side of the tube.
14. Add the collected 40% layer to a 15mL tube and fill to 15mL with PBS. Mix by inversion.
15. Transfer the mixture into an Amicon Ultra-15 100kDa centrifuge tube. Centrifuge at 1900 x g for 15 min at 4 deg.
16. Discard flow-through and add 14mL of PBS to top of centrifuge tube. Mix by inversion. Centrifuge again at 1900 x g for 15 min at 4 deg.
17. Repeat step 16. To obtain higher titers, the length of centrifugation can be increased.
18. Collect final volume (typically 50-200uL) of concentrated AAV vector. Aliquot and store at -80 deg.

Buffers:

- Lysis buffer (100mL): 3mL 5M NaCl + 2mL 1M tris + 95mL ddH2O
- Iodixanol solution A (50mL, colorless): 5mL 10x PBS + 50uL 1M MgCl2 + 125uL KCl + 10mL 5M NaCl + 12.5mL OptiPrep + 22.3mL ddH2O
- Iodixanol solution B (50mL, red): 5mL 10x PBS + 50uL 1M MgCl2 + 125uL KCl + 20mL OptiPrep + 100uL phenol red + 24.7mL ddH2O
- Iodixanol solution C (50mL, colorless): 5mL 10x PBS + 50uL 1M MgCl2 + 125uL KCl + 33.3mL OptiPrep + 11.5mL ddH2O
- Iodixanol solution D (50mL, yellow): 50uL 1M MgCl2 + 125uL KCl + 50mL OptiPrep + 25uL phenol red

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wang, S. K. and Cepko, C. L.(2021). AAV vector production. Bio-protocol Preprint. bio-protocol.org/prep1211.
2. Wang, S. K., Xue, Y. and Cepko, C. L.(2020). Microglia modulation by TGF-β1 protects cones in mouse models of retinal degeneration. The Journal of Clinical Investigation 130(8). DOI: 10.1172/JCI136160